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WO 92/22635 A1
Transgenic Research 1994, 3(3), pp.141-151 - Barash et al.

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Online: EPODOC, WPI, BIOSIS, MEDLINE, CAS-ONLINE, SCISEARCH, EMBASE

(54) Abstract Title
Human serum albumin expression and use in gene therapy

(57) The present invention relates to DNA vectors as a means of gene therapy for the use in treatment of human and animal disease states that are characterised by failure or inadequate functioning of the liver. This results in the lowering of the blood levels of certain important proteins produced by the liver leading to hypoalbuminaemia, anaemia, thrombocytopenia and coagulation disorders related to liver failure. The invention also relates to an expression cassette for expressing a human serum albumin into a mammalian cell comprising:
(i) a cDNA sequence of the human serum albumin,
(ii) a transcriptional control sequence operably linked to said cDNA sequence.

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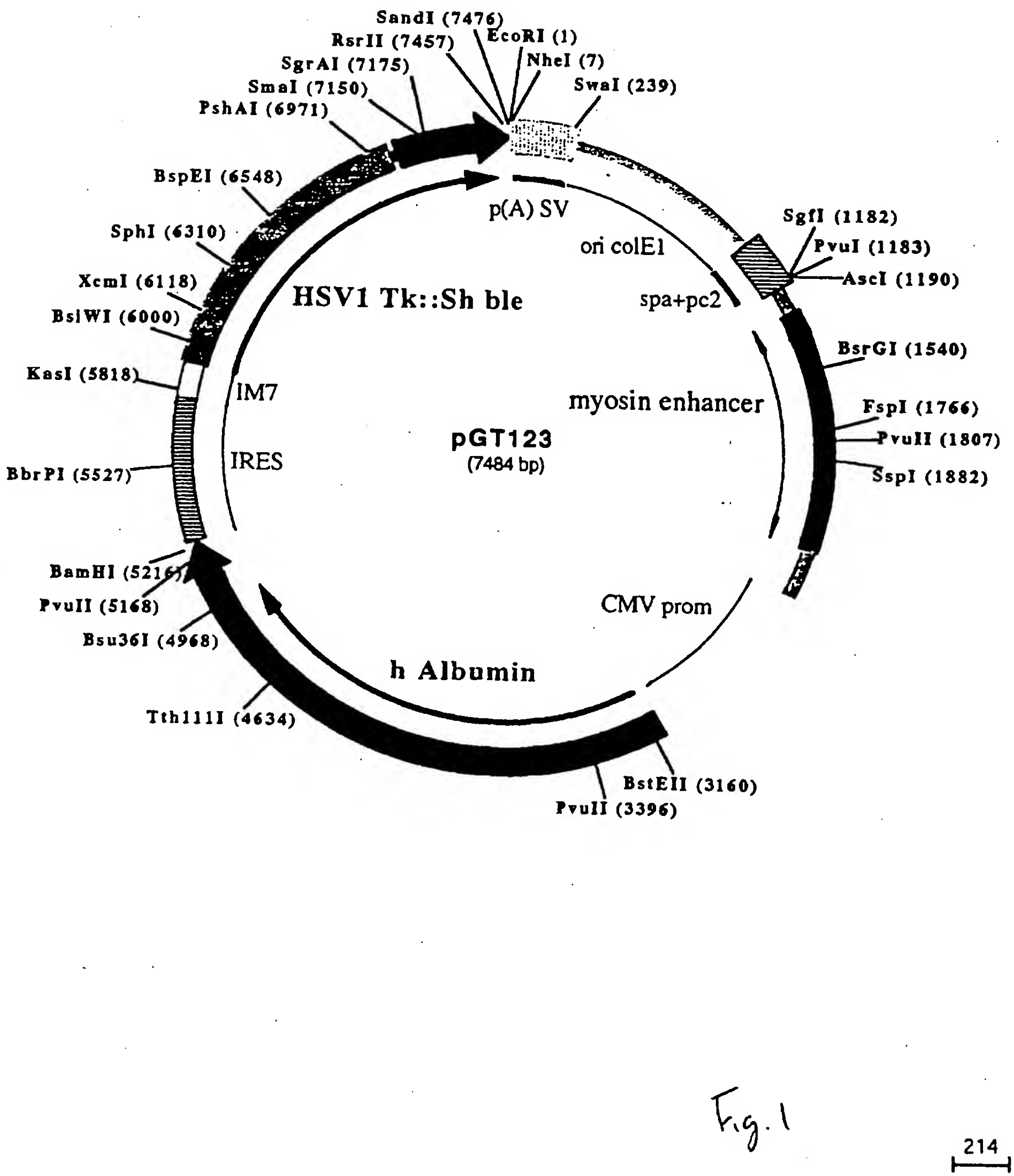
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SphI (6310)
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PvuI (1183)
XcmI (6118)
AscI (1190)
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BsiWI (6000)
BsrGI (1540)
KasI (5818)
IM7
myosin enhancer
FspI (1766)
pGT123
(7484 bp)
PvuII (1807)
IRES
BbrPI (5527)
SspI (1882)
BamHI (5216)
CMV prom
PvuII (5168)
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h Albumin
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BstEII (3160)
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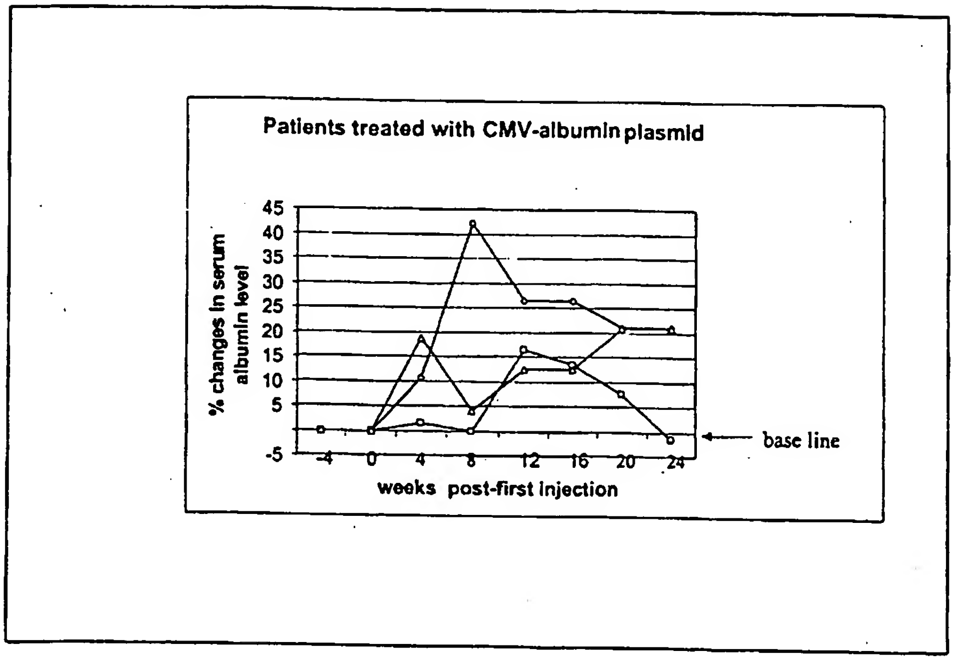
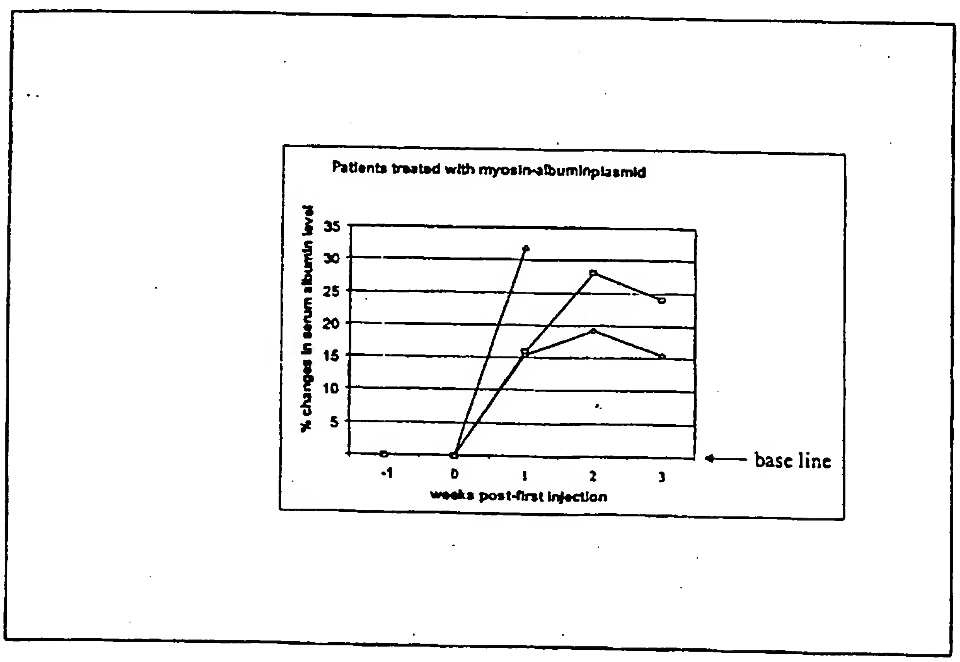
214

Fig. 1

214



Patients treated with CMV-albumin plasmid
% changes in serum albumin level
45
40
35
30
25
20
15
10
5
-5
-4
0
4
8
12
16
20
24
weeks post-first injection
base line



Patients treated with myosin-albuminplasmid
% changes in serum albumin level
35
30
25
20
15
10
5
-1
0
1
2
3
weeks post-first injection
base line

Fig 2

Changes in serum albumin level

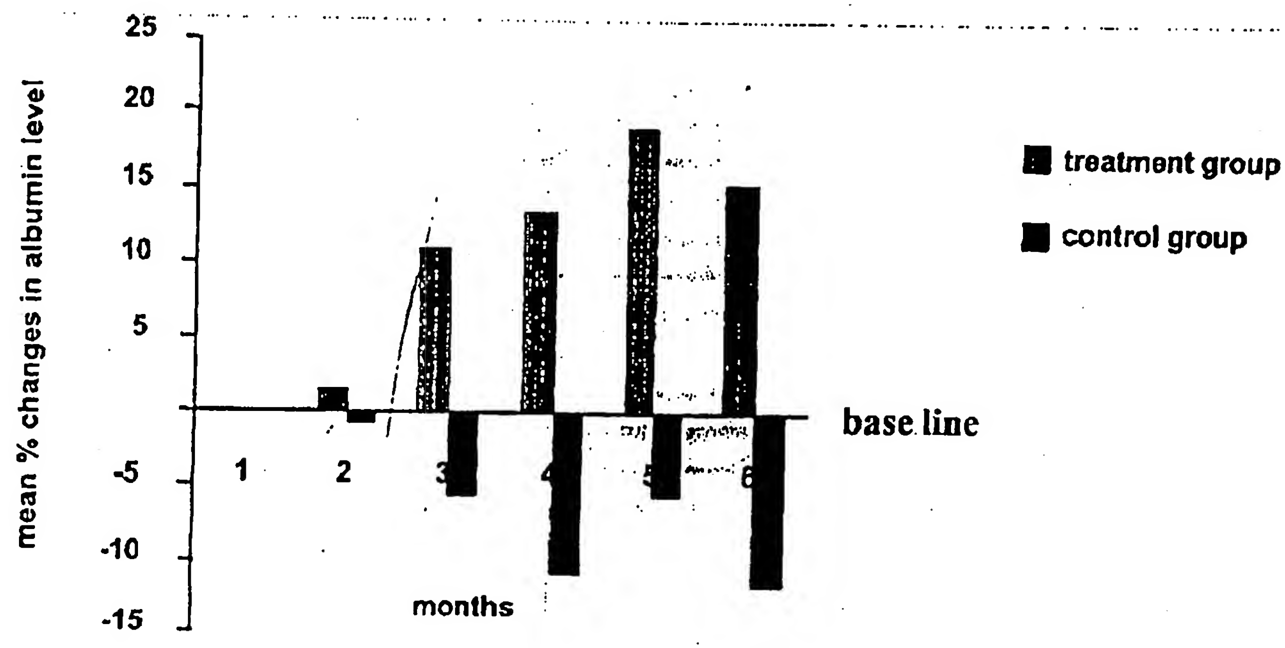


Fig 3

4/4

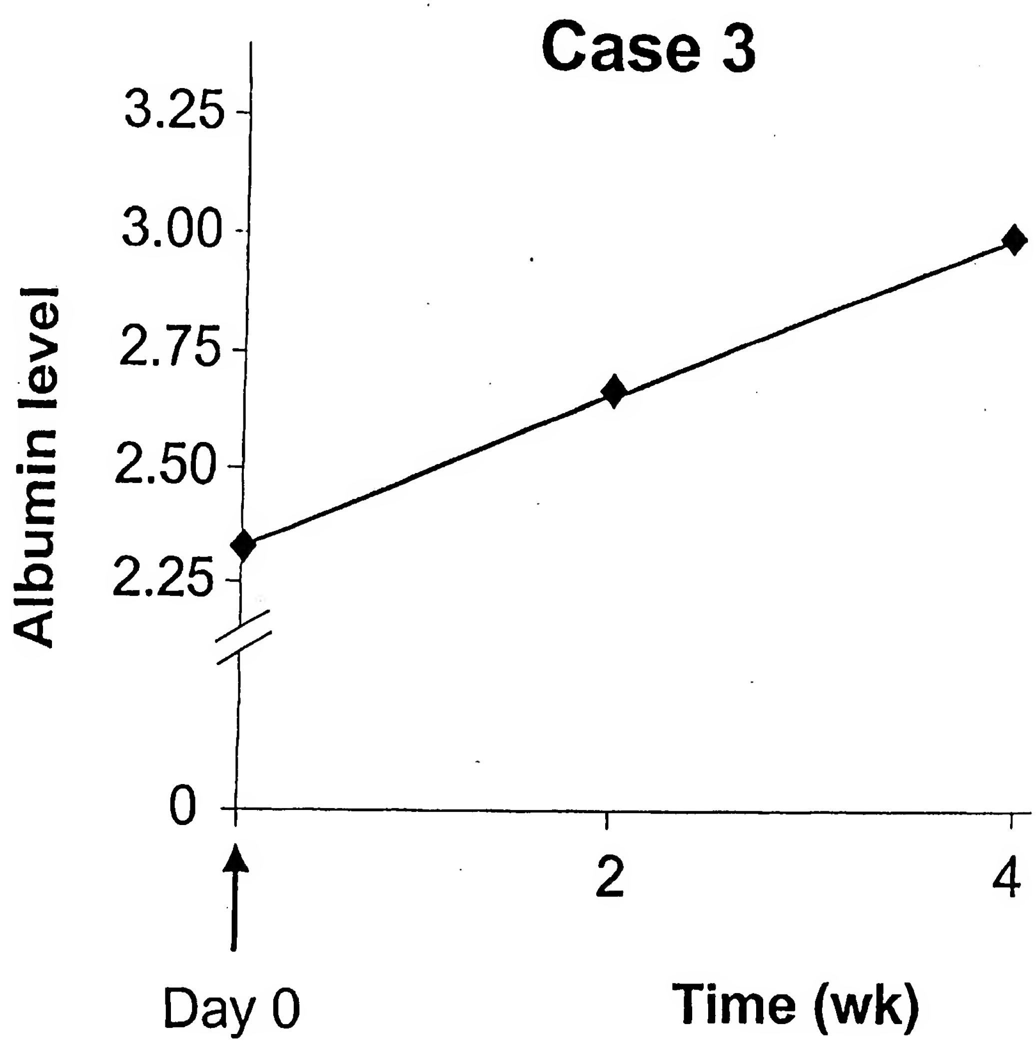


Fig 4

A METHOD FOR PROMOTING EXTRA-HEPATIC PRODUCTION OF PROTEINS FOR THE CORRECTION OF HYPOALBUMINAEMIA, ANAEMIA, THROMBOCYTOPENIA AND/OR COAGULATION DISORDERS RELATED TO LIVER FAILURE.

The present invention relates generally to the fields of human treatment of complications and side-effects disorders associated with a failure of the liver to produce proteins and albumin in particular, and more specially to expression cassettes and related recombinant DNA compounds useful in correction of low serum albumin (hypoalbuminaemia) by gene therapy.

Human serum albumin (HSA) is a naturally-occurring, globular, nonglycosylated polypeptide (MW 65,000) comprising nearly 600 amino acid residues (see e.g. Lawn et al., 1981, Nucleic Acid Research, 9, 6103-6113) synthesized in the liver of higher species. This protein plays important roles in the organism as it the maintenance of osmotic pressure; is involved in the binding and transport of a wide variety of substances such as fatty acids, ions (copper, nickel, calcium), bile, bilirubin, protoporphyrin, prostaglandins, steroid hormones, thyroxine, cystine, glutathione, various drugs and water soluble vitamins and the like; and additionally provides a direct source of amino acids to tissues.

In healthy conditions HSA is the most abundant protein found in the plasma of adult humans where its concentration is about 40mg/ml. However, this level can dramatically decline in the context of many diseases, especially those affecting the liver, such as for example hepatitis, cirrhosis, primary and secondary liver cancers, or infectious and inflammatory conditions, chemotherapy and radiotherapy treatments, malabsorption and nephrotic syndromes (for a medical revue, see Doweiko et al., 1991, J. Parenteral and Enteral Nutrition, 15, 476-483) which are generally associated with a reduced synthesis of albumin or an excessive metabolization of said protein leading to plasma low concentrations and to complications or side effects such as hypovolemia, hypoproteinemia, ascites formation, oedema, etc... Moreover, Goldwasser and Feldman (1997, J. Clin. Epidemiol, 50, 693-703) have reported an association between hypoalbuminaemia and mortality risk. These authors have particularly shown an increase in the odds of death ranges from 24% to 56% for each 2.5mg/ml decrement in serum albumin concentration. HSA concentrations below 35 mg/ml indicates a pathological status. The method described in the invention is a platform technology with applications for the treatment and correction of various human and animal disease states. Hypoalbuminaemia is not a single disease, but can occur as a consequence of many diseases. Therefore the invention is not restricted in its use to the treatment of a single condition. Hypoalbuminaemia can occur if the liver has an underlying disease state and is failing to produce adequate amounts of albumin. This occurs in diseases such as hepatitis, cirrhosis and liver cancer (both primary and secondary cancers). Hypoalbuminaemia also occurs as the result of

any disease state that affects the body's metabolism, such as advanced cancer, the treatment of cancer with chemotherapy or radiotherapy, catabolic states such as severe infections or inflammation, malabsorption or the nephrotic syndrome and recovery from major surgery. All of these conditions, and many others, can result in a reduced level of albumin in the blood and would be suitable for treatment using the method described in this invention. In addition, the invention could be used to provide a source of other proteins, in addition to albumin, produced at sites other than the physiological source of the proteins in the body. In this invention the prime extra site of production is the skeletal muscle, but other sites could be included. The other proteins that could be used in this invention include, but are not restricted to, erythropoietin for the correction of anaemia, thrombopoietin for the correction of of thrombocytopaenia, protein C and protein F for patients requiring anticoagulation, and leptin for the treatment of obesity. In addition, the invention can be used to produce hormones and growth factors used by the human body at sites other than the physiological source of such hormones and growth factors. These would include but not restricted to insulin for the treatment of diabetes, endothelial nitric oxide synthetase (ENOS) for the prevention of thrombosis after coronary artery surgery or coronary balloon dilatation or myocardial ischaemia, Granulocyte Colony Stimulating Factor (GCSF), Macrophage Colony Stimulating Factor (MCSF) or Granulocyte Macrophage Colony Stimulating Factor (GMCSF), and/or interferon for the treatment of hepatitis or cancer.

Careful treatment of hypoalbuminaemia is a key to improving quality of life and to reducing mortality risk of affected patients. The standard treatment of hypoalbuminaemia is the administration of albumin employed as a therapeutic drug.

First, commercial HSA has been prepared from human plasma as a by product from the fractionation of donated blood. However, availability of human plasma is limited and careful heat treatment of the product prepared from human plasma should be effected to avoid potential contamination of the HSA product by hepatitis ~ and HIV viruses. Moreover, said isolation of HSA from natural sources is technically difficult, expensive and time consuming.

Against this background related to consideration of ethics, safety and stability of supply, further efforts have centered on the development of efficient recombinant methods for the production of recombinant HSA in bacterial cells or yeast which could be used as a substitute for natural HSA (Hone et al., 1998, Gen. Pharmac., 31, 811-815).

Although effectiveness of the exogenous albumin supply to the patient has been supported, indicating that serum level can be transiently restored, said treatment still means that repeated administration of albumin is required every 48 hours throughout the lifetime of the patient.

Accordingly,the prior art is deficient in providing a satisfactory treatment method providing an acceptable level of serum albumin and being compatible with quality of life of the patients. The present invention fulfills this longstanding need and desire in the art.

Since the discovery that skeletal muscle can be transfected in *vivo* by transmuscular injection of plasmid DNA, this organ system has attracted considerable attention as a potential source of secreted therapeutic proteins. However, the efficiency of this method of transfection is still low, even with the induction of muscle degeneration and regeneration through injection of myotoxic substances prior the injection of DNA. Accordingly, most studies so far have shown that expression is not high enough to increase the blood levels of circulating proteins, especially in case where this level should be high enough for permitting an improvement of the health.

It was now surprisingly found that the transfection of a polynucleotide comprising the cDNA sequence of human serum albumin into vertebrate tissue leads to a dramatic expression of HSA which provides an efficient tool for correcting hypoalbuminaemia and associated disorders. In particular, it was surprisingly found that injection into muscular tissue of said polynucleotide leads to a significant augmentation of serum albumin levels for up to three months and to reduced complications or side effects in treated patients. Thus, the present invention first relates to an expression cassette, reliable recombinant DNA compound and their use for the preparation of a pharmaceutical composition which provide a steady constitutive level of expression allowing an effective amount of protein to be produced and a restoring in the treated patients albumin levels leading to improved clinical status. Generally, the present invention provides a gene therapy approach aiming to increase the serum albumin level at least to the lower value of the normal range.

Therefore, the technical problem underlying the present invention is the provision of improved methods and means for the correction of hypoalbumineamia by gene therapy. This technical problem is solved by the provision of the embodiments as defined in the claims.

The present invention concerns an expression cassette for expressing a human serum albumin into a mammalian cell comprising:

(i) a cDNA sequence of the human serum albumin,

(ii) a transcriptional control sequence operably linked to said cDNA sequence.

According to a preferred embodiment, said expression cassette comprises a cDNA sequence of the human serum albumin having the nucleotide sequence set forth in SEQ ID NQ:1

Said expression cassette can also be constructed by using a cDNA sequence of the human serum albumin of about 2.Okb in length obtained by enzymatic digestion by Kpnl and Sal1 of the plasmid PILMALB5 having the deposit accession number HSRRB HG226.

It should be also noticed here that the cDNA production from mRNA is well described in the literature and that two cDNA sequences could be obtained from a unique mRNA depending on the oligonucleotide sequences used in order to initiate the reverse transcription of said mRNA. Said cDNA sequences differ only at their respective 5'end and/or 3'end where sequence variations can occur. Nevertheless, each of said cDNA sequences will comprise the correct sequence corresponding to said mRNA and accordingly can be used in the context of the present invention.

"operably linked" means that the cDNA and the transcriptional control sequences are in a relatioship permitting them to function in their intended manner. Thus, for example, a promoter operably linked to a cDNA sequence is ligated in such a way that expression of the human serum albumin is achieved under conditions which are compatible with the transcriptional activaty of the promoter. These conditions are widely used in the technical field of the invention.

"Expression of a gene, and therefore of the corresponding polypeptide", means that the encoding DNA sequence is first transcribed in mRNA, which itself is further translated in the corresponding polypeptide seouence.

"Human serum albumin or HAS" according to the present invention preferably designates a polypeptide sequence as set forth in SEQ ID N:2 (Lawn) - However, comparison analysis of published HSA sequences (Lawn et al., 1981, Nucleic Acid Res., 9, 6103-6114 and Dugaiczyk et al., 1982, PNAS, 79, 71-75 for example) shows that minor amino acid variations are acceptable in the HSA polypeptide sequence which do not affect the HSA properties. Accordingly, and with regard to the degenerescence of the genetic code, the skilled man can easily adapt the cDNA sequence of the present invention with respect to these minor changes. These specific embodiments are encompassed by the invention.

"Transcriptional control sequence" designates the nucleic acid sequences which control the initiation of the transcription, the selection of the start position, which regulate the transcription level (enhancement or inhibition), which determine the type of polymerase directing the polymerisation of the transcribed mRNA, which control the transcription rate, the termination of said transcription, and the site of said termination. These sequences are widely analyzed, used and reported in the literature and can be readily obtained or adapted by those skilled in the art.

In a specific embodiment, the transcriptional control sequence according to the invention comprises a promoter element which is selected from the group consisting of viral promoters. Examples of such viral promoters are the SV4O early and late promoters, the adenovirus major late

promoter, the Rous Sarcoma Virus (RSV) promoter, the Cytomegalovirus (CMV) immediate-early promoter, the herpes simplex virus (HSV) promoter, MPSV promoter, 7.5k promoter, vaccinia promoter and the Major-intermediate-early (MIE)promoter. The Cytomegalovirus (CMV) immediate-early promoter is however preferred.

The promoter nucleic acid sequence can be a naturally occurring promoter isolated from biological nucleic acid material or chemically synthesised. The promoter sequence can also be artificially constructed by assembling elements previously screened for transcriptional activity leading to potencies which can exceed those of naturally occuring ones (Li et al., 1999, Nature Biotech., 17, 241-245)

According to the present invention, "nucleic acid sequence" means a fragment or a portion of a nucleic acid, without size limitation, which may be either linear or circular, natural or synthetic, modified or not (see US 5525711, US 4711955 US 5792608 or EP 302175 for modification examples) . Depending on the considered sequence, it may be, *inter alia,* a genomic DNA, a cDNA or a synthesised DNA. More elaborated "nucleic acid sequences" such as "recombinant DNA compound" may be in the form of linear nucleic acid construct, and preferably in the form of plasmid. According to the invention, said "recombinant DNA compound" should preferably be understood as a naked nucleic acid construct or " plasmid vector" (Wolff et al., Science 247 (1990), 1465-1468), or as nucleic acid construct formulated with at least one compound such as polypeptides, preferably viral polypeptides (for a review, see Robbins et al., 1998, Tibtech, 16, 35-40) (said "recombinant DNA compound" is herein termed " viral vector") , or cationic lipids or cationic polymers which can participate in the uptake or stabilization of the nucleic acid construct into the cells (see Ledley, Human Gene Therapy 6 (1995) , 1129-1144 or Rolland, 1998, Therapeutic Drug Carrier Systems, 15, 143-198 for a review) (said "recombinant DNA compound" is termed " synthetic vector") . Accordin9 to the invention, the nucleic acid sequence can be homologous or heterologous to the host expressing cells.

The expression cassette can be constructed using routine cloning techniques known to persons skilled in the art (for example, see Sambrook et al., 1989, Molecular Cloning-a laboratory manual ; Cold Spring Harbor Press).

In still another aspect of the invention, the transcriptional control sequence further comprises at least one enhancer element. The definition of enhancers refers to regulatory elements which activate transcription in a position and orientation independent way. Several enhancer elements have been identified to date in many genes. Preferably, the present enhancer element is a myosin light chain enhancer. More preferably, the enhancer used in the expression cassette of the present invention is of vertebrate origin, more preferably of mammalian origin. The rat myosin light chain 1/3 enhancer

(Donoghue et al., 1988, Gene & Dev., 2, 1779-1790) is especially preferred. The enhancer element is operably linked to the promoter, may be localized either upstream or downstream of said promoter and may be used in either orientation. In a specific construction of the present invention, said enhancer sequence is located upstream of said cDNA sequence of the human serum albumin. According to a preferred embodiment, said transcriptional control sequence comprises at the most three enhancer sequences, the sequence of which is identical or selected independently of one another.

The present invention is more specifically directed to an expression cassette comprising the sequence set forth in SEQ ID NQ:5 which codes for said myosin light chain 1/3 enhancer, said CMV promoter and said cDNA sequence of the human serum albumin.

In general, the transcriptional control sequence further comprises at least one sequence responsible for the polyadenylation of the transcribed RNA molecules. Said sequence may be selected in the group consisting of bGH (bovine growth hormone) polyadenylation signal (EP 173552), SV40 polyadenylation signal, globine polyadenylation signal.

The invention also concerns an expression cassette further comprising a polynucleotide sequence encoding at least one polypeptide of interest which is distinct from human serum albumin, said polypeptide of interest being co-expressed with human serum albumin in said mammalian cell. It further concerns an expression cassette wherein said polynucleotide sequence is localized downstream of said cDNA sequence, is operably linked to the same transcriptional control sequence thereof and said cDNA and polynucleotide sequences being linked by an additional sequence comprising at least one ribosome binding site that facilitates translation of the encoded messenger. According to a preferred embodiment, said polypeptide of interest is a selectable marker such as an antibiotic resistance polypeptide or an hybrid polypeptide thereof. In a preferred embodiment, said antibiotic is bleomicin or the polynucleotide sequence encoding said hybrid is an hybrid gene bleomicin:thymidine kinase. The thymidine kinase gene encodes a product which is able in conjunction with a pro-drug such as gancyclovir (GCV) to kill cells expressing said gene product.

From yet another perspective, the invention includes a recombinant DNA compound comprising at least one of the expression cassette above described. Said recombinant DNA compound can further include at least one nucleotide sequence containing or expressing targeting sequences, transport sequences, sequences involved in replication or integration, or sequence encoding a selectable marker, for example for antibiotic resistance (ampicilin, phleomycin, chloramphenicol), useful for selecting a cell in which said compound has been introduced. Example of such sequences have been reported in the literature and can be readily obtained by those skilled in the art. The recombinant DNA compound can also be modified in order to be stabilized with specific components such as spermine.

As previously stated, the recombinant DNA compound of the invention may be a vector selected from the group consisting of plasmid vector, viral vector or synthetic vector.

The invention further concerns a host cell transformed with a said recombinant DNA compound. Said host cell is preferably a mammalian cell, and more preferably a human muscular cell. This cell can originate from various tissues including those of muscle, skin, nose, lung, liver, spleen, bone marrow, thymus, heart, lymph, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, connective tissue, blood, tumor etc.

According to the invention, "transformed" means either transfection or infection, and more broadly designate any transferring step resulting in introduction of said recombinant DNA compound in said host cell. Said transferring step can be implemented by any of a wide variety of ways, including method selected from the group consisting of adenoviral infection, transfection with nucleic acid coated particles such as lipoplexes (cationic lipid /nucleic acid complexes) or polyplexes (cationic polymer Inucleic acid complexes) or the like, calcium phosphate transfection of plasmid, transfection with naked nucleic acid, electroporation method or any combination thereof. However, the particular method for introducing the foreign nucleic acid sequence is not crucial to the invention.

Moreover according to a specific embodiment, said transformed host cell is a human muscle and is further encapsulated. Cell encapsulation methodology has been previously described which allows transplantation of encapsulated cells in treatment of Parkinson's disease (Tresco et al., 1992, ASAIO J., 38, 17-23) or Amyotrophic lateral sclerosis (Aebischer et al., 1996, Hum. Gene Ther. , 7, 851-860). According to said specific embodiment, transformed cells are encapsulated by compounds which form a microporous membrane, and said encapsulated cells can further be implanted *in vivo*. Capsules, for example approximately 1 cm in length containing the cells of interest may be prepared employing a hollow microporous membrane fabricated from poly-ether-sulfone (PES) (Akzo Nobel Faser AG, Wuppertal, Germany; Deglon et al, 1996, Hum. Gene Ther., 7, 2135-2146). This membrane has a molecular weight cutoff greater than 1,000,OOODa, which permits the free passage of proteins and nutrients between the capsule interior and exterior, while preventing the contact of transplanted cells with host cells. The entrapped cells may be implanted by intradermal, subdermal, intravenous, intramuscular, intranasal, intracerebral, intratracheal, intraarterial, intraperitoneal, intravesical, intrapleural, intracoronary or intratumoral ways. In case where said transformed host cell is a myoblast, it can migrate from the site of injection to muscles where expression of human serum albumin can occur.

The present invention concerns the specific use of a cDNA sequence of the human serum

albumin, of an expression cassette, of a recombinant DNA compound or of a transformed host cell as previously disclosed for the preparation of therapeutic composition intended for gene transfer, especially for the treatment of the human or animal body. Administration into vertebrate target tissues, and more specifically into the muscle, can be performed by different delivery routes (systemic delivery and targeted delivery) . According to the present invention, the prepared therapeutic composition is preferably administered into muscle, however prepared therapeutic composition administration can also occur in other tissues of the vertebrate body including those of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, connective tissue, blood, tumor. The human serum albumin can thus be excreted in body fluids (eg. blood) allowing its delivery in patient organs or said polynucleotide can be associated with targeting molecules which are capable to point its uptake into targeted cells. Gene therapy literature provides many mechanisms for efficient and targeted delivery and expression of genetic information within the cells of a living organism (see for example European patent application n°98/401108.0) . Said administration may be made by intradermal, subdermal, intravenous, intramuscular, intranasal, intracerebral, intratracheal, intraarterial, intraperitoneal, intravesical, intrapleural, intracoronary or intratumoral injection, with a syringe or other devices. Transdermal administration is also contemplated, as are inhalation or aerosol routes. Intramuscular administration route is preferred.

The present invention allows repeated administrations to the patient without major risk of the administered preparation to induce a significant immune reaction. Administration may be by single or repeated dose, once or several times after a certain period of time. Repeated administration allows a reduction in the dose of recombinant DNA compound administered at a single time. The route of administration and the appropriate dose vary in function of several parameters, for example the individual patient, the side effects of the disease, or the albumin level before treatment.

In general, the concentration of polynucleotide in the pharmaceutical compositions is from about 0.1 μg/ml to about 20 mg/ml.

The active dose, or the amount of recombinant DNA compound which should be injected for obtaining satisfactory serum albumin levels, is from about 1 mg to about 50 mg, preferably from about 2 mg to about 24 mg. Said active dose can be administered in a unique administration or in multiple ones distributed into one or more days. For example, the administration cycle is composed of three days treatment : injection of 2 to 8 mg the first day, 2 to 8 mg the second day and 2 to 8 mg the third day. Preferably, the maximum single dose is 8 mg of DNA administered. The separate administrations can be performed by different delivery routes (systemic delivery and targeted delivery, or targeted deliveries for example) . In a preferred embodiment, each should be done into the

same target tissue and most preferably by injection.

In a further preferred embodiment the invention pertains to a pharmaceutical preparation comprising at least one of the complexes described above and also incorporating at least one adjuvant capable of improving the transfection capacity of said complex or gene expression into cell and selected from the group consisting of chloroquine, protic compounds such as propylene glycol, polyethylene. glycol, glycerol, ethanol, 1-methyl L-2-pyrrolidone or derivatives thereof, aprotic compounds such as dimethylsulfoxide (DM50), diethylsulfoxide, di-n-propylsulfoxide, dimethylsulfone, sulfolane, dimethyl-formamide, dimethylacetamide, tetramethylurea, acetonitrile or derivatives. The composition may also advantageously comprise a source of a cytokine which is incorporated in the form of a polypeptide or as a polynucleotide encoding the cytokine. Preferably, said cytokine is interleukin 10 (IL-b) (see European patent application, filing number 98 40 1667.5 or related foreign application). The therapeutic composition can further comprise an nuclease inhibitor such as actine G (see European patent application, filing number 98 40 1108.0 or related foreign application), or specific magnesium concentration (see European patent application, filing number 98 40 2424.0 or related foreign application)

Therefore, the invention further provides a pharmaceutical composition comprising the above cited recombinant DNA compounds which may also comprise a pharmaceutically acceptable injectable carrier. The carrier is preferably isotonic, hypotonic or weakly hypertonic and has a relatively low ionic strength, such as provided by a sucrose solution. It includes any relevant solvant, aqueous or partly aqueous liquid carrier comprising sterile, pyrogen-free water, dispersion media, coatings, and/or equivalents. The pH of the pharmaceutical preparation is suitably adjusted and buffered.

In the case of *in vivo* treatment according to the invention, in order to improve the transfection rate, the patient may undergo a macrophage depletion treatment prior to administration of the pharmaceutical preparations described above. Such a technique is described in the literature (refer particularly to Van Rooijen et al., 1997, TibTech, 15, 178-184). The patient can also be pre-treated with prednisolone or equivalent.

The invention concerns a method for treating a disorder associated with hypoalbuminaemia in a mammalian organism comprising the step of transferring a recombinant DNA compound into tissue or cells of said organism, wherein said recombinant DNA compound comprises an expression cassette recited in the present invention and such that human serum albumin is expressed resulting in the treatment of said disorder. However, the methods described in this invention can also be applied to the treatment of other conditions and diseases of the human

and animal body in which there is a failure or relative failure of the liver to produce proteins or sufficient quantities of the said proteins to maintain normal function o the tissues or body. Such proteins would include coagulation factors, thrombopoietin. The methods described in the invention would also apply to conditions or diseases of the human or animal body in which there is a failure of the body or organs of the body to produce sufficient hormones, enzymes, growth factors, cytokines or other substances necessary for normal function or homeostasis. The other proteins that could be used in this invention include, but are not restricted to, erythropoietin for the correction of anaemia, thrombopoietin for the correction of of thrombocytopaenia, protein C and protein F for patients requiring anticoagulation, and leptin for the treatment of obesity. In addition, the invention can be used to produce hormones and growth factors used by the human body at sites other than the physiological source of such hormones and growth factors. These would include but are not restricted to insulin for the treatment of diabetes, endothelial nitric oxide synthetase (ENOS) for the prevention of thrombosis after coronary artery surgery or coronary balloon dilatation or myocardial ischaemia, Granulocyte Colony Stimulating Factor (GCSF), Macrophage Colony Stimulating Factor (MCSF) or Granulocyte Macrophage Colony Stimulating Factor (GMCSF), and/or interferon for the treatment of hepatitis or cancer.

The following examples illustrate the invention.

Example 1

Case Nos 1, 2 and 3 demonstrate the rise of serum albumin in 3 patients following the administration of plasmid encoding albumin. Each patient received 1.5mg intra-muscular DNA daily for 4 consecutive days.

Case 1: A middle-aged male patient with long-standing cirrhosis due to hepatitis. The patient had marked ascites and a serum albumin level of 1.9mmol/l (normal >3.2mmol/l). The DNA/Promotor was administered once daily, at a dose level of 1.5mg, for four consecutive days. The patient was receiving no additional therapy. Serum albumin level rose to 2.7mmol/l within three weeks and this level was sustained for ten weeks. After sixteen weeks the serum albumin level had fallen to 2.3mmol/l. During this period the ascites improved significantly.

Case 2: An elderly male patient with hepatocellular carcinoma resulting from hepatitis and cirrhosis. The patient was not receiving any therapy for the condition. The serum albumin pre-treatment was 3.0mmol/l. Intra-muscular injections of DNA/Promotor were given, as in case 1. Serum albumin levels rose to 3.9mmol/l within three weeks and remained at normal levels for three months.

Case 3: A patient with cirrhosis due to hepatitis C infection. The patient was receiving diuretics (aldactone and lasix) as treatment for ascites. Pre-treatment the patient's serum albumin level was 2.9mmol/l. This level rose to 3.7mmol/l after the course of treatment with the DNA/Promotor. This was accompanied by a significant improvement in the ascites.

SEQ ID NO:1

11

NheI (7)
EcoRI (1)
1 GAATTCGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTT

80 TATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGC

159 ATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAGAT

SwaI (239)
238 GATTTAAATGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCG

317 TTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC

396 TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCT

475 GTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTT

554 CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT

633 CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGG

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791 CCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTT

870 GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG

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PvuI (1
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1107 GACCCCACTGGACAAACAGTGGCTGGACTCTGCACCATAACACACAATCAACAGGGGAGTGAGCTGGATCGAGCTGCGA

AscI (1190)
1186 TCGCGGCGCGCCACTAGTTAACTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC
1265 GCACATTTCCCCGAAAAGTGCCACCTGACGTCGACGGATCGGGAGATCCGCTATTAATCCCAGAGCCCTTGGAAGCCAG

1344 AGGAAGATGTATCTCTGAGTTTGAGGCTACCCTACTCTACAGAAAGAGTTCCAGGACAGACATTACACGAGAAGCCCTG

1423 CCCCCTCTCTAAAATAAAAGTATTTTCAGAAGCATAAAGGTCACAGTGTAGAGAAAATGACTGCTACACGTAGTCTTAA

BsrGI (1540)
1502 TTATAGAGGGCTCTTTTTTTTTTTTTTTTGATCTGTGGTGTACATGTCTTTACATTTTTTTCAAGATAGAAAAGCATGA

1581 TGTCTGTGCGGTATAAATTGTTCGTTTTGAGCCTTGTGTATAACGCTTTCCTCTCAAGATTTTATAATAGTGCTTTAAC

1660 TGTCCCCACGGGCTAACTTCAGCACACTGTCATGGGACCTAACCTTATTAAATTACCATGTGTGAACCGCTCATAACTC

FspI (1766)
PvuII (1807)
1739 AAGTCGCAGCAGGTGCAAAAATGGAGCTGCGCAGGCAGAAGAGTGATCGTCATTTTTAAAATCCCCACCAGCTGGCGAA

SspI (1882)
1818 GCAACAGGTGCCTAATTCCTCATCTTTTAAAAATAACTTTTCAAAAGCCTGTGCTGTATAAGCAAATATTTTCAAGTTT

1897 GTTTTTAAACCATCTTCAAGTTACCTTTCCTCACAAAATACATTATGTGCTGATTTTTTTGTCTCAAAATGACATTTGA

12

1976 AGTCTAAGCATATAAAAATTTATTTCTTTTTAGAAATGAAATTATTATTTAACTGGAGACTTAAATTGTGTCTTAACTC

2055 TTGCTCCTCCCCTTTTCCCCTTTTGTCCCTTCTCTCCCCACTCCCCTCCCCTTCTCTTCACATGCTCATGGCGGGCTCT

2134 TCTCTTTCCTACTCTTCTTCTTTCTCTCATCCCTCTCCCTTGTCTTGTCCTTTCACTAAACCTTTCCACATGGAAAAAA

2213 TAAATTGTATCTTAAAGCTCGGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT

2292 AAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAA

2371 GGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCG

2450

2529

2608

2687

2766

2845

2924

3003

BstEI

3082 CTCTTCTGTCAACCCCACGCCTTTGGCACAATGAAGTGG

1▶ MetLysTrp

3161 GTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCCAGGGGTGTGTTTCGTCGAGATGCACACAAGAGTG

4▶ ValThrPheIleSerLeuLeuPheLeuPheSerSerAlaTyrSerArgGlyValPheArgArgAspAlaHisLysSerG

3240 AGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCA

30▶ luValAlaHisArgPheLysAspLeuGlyGluGluAsnPheLysAlaLeuValLeuIleAlaPheAlaGlnTyrLeuGl

PvuII

3319 GCAGTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTAGCTGATGAGTCA

56▶ nGlnCysProPheGluAspHisValLysLeuValAsnGluValThrGluPheAlaLysThrCysValAlaAspGluSer

3398 GCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGAAACCTATG

83▶ AlaGluAsnCysAspLysSerLeuHisThrLeuPheGlyAspLysLeuCysThrValAlaThrLeuArgGluThrTyrG

3477 GTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGATGACAACCCAAA

109▶ lyGluMetAlaAspCysCysAlaLysGlnGluProGluArgAsnGluCysPheLeuGlnHisLysAspAspAsnProAs

3556 CCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAAA

135▶ nLeuProArgLeuValArgProGluValAspValMetCysThrAlaPheHisAspAsnGluGluThrPheLeuLysLys

3635 TACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTG

162▶ TyrLeuTyrGluIleAlaArgArgHisProTyrPheTyrAlaProGluLeuLeuPhePheAlaLysArgTyrLysAlaA

3714 CTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAA

188▶ laPheThrGluCysCysGlnAlaAlaAspLysAlaAlaCysLeuLeuProLysLeuAspGluLeuArgAspGluGlyLy

3793 GGCTTCGTCTGCCAAACAGAGACTCAAATGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTG

214▶ sAlaSerSerAlaLysGlnArgLeuLysCysAlaSerLeuGlnLysPheGlyGluArgAlaPheLysAlaTrpAlaVal

3872 GCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACA

241▶ AlaArgLeuSerGlnArgPheProLysAlaGluPheAlaGluValSerLysLeuValThrAspLeuThrLysValHisT

3951 CGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAGGA

267▶ hrGluCysCysHisGlyAspLeuLeuGluCysAlaAspAspArgAlaAspLeuAlaLysTyrIleCysGluAsnGlnAs

4030 TTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAAT

293▶ pSerIleSerSerLysLeuLysGluCysCysGluLysProLeuLeuGluLysSerHisCysIleAlaGluValGluAsn

4109 GATGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGG

320▶ AspGluMetProAlaAspLeuProSerLeuAlaAlaAspPheValGluSerLysAspValCysLysAsnTyrAlaGluA

4188 CAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAG

346▶ laLysAspValPheLeuGlyMetPheLeuTyrGluTyrAlaArgArgHisProAspTyrSerValValLeuLeuLeuAr

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 67 ACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTC
 372▶ gLeuAlaLysThrTyrGluThrThrLeuGluLysCysCysAlaAlaAlaAspProHisGluCysTyrAlaLysValPhe
4346 GATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAACTGTGAGCTTTTTAAGCAGCTTGGAGAGT
 399▶ AspGluPheLysProLeuValGluGluProGlnAsnLeuIleLysGlnAsnCysGluLeuPheLysGlnLeuGlyGluT
4425 ACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTAGAGGTCTC
 425▶ yrLysPheGlnAsnAlaLeuLeuValArgTyrThrLysLysValProGlnValSerThrProThrLeuValGluValSe
4504 AAGAAACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGAAGACTATCTA
 451▶ rArgAsnLeuGlyLysValGlySerLysCysCysLysHisProGluAlaLysArgMetProCysAlaGluAspTyrLeu
                                                          Tth111I (4634)
4583 TCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACAAAATGCTGCACAGAGT
 478▶ SerValValLeuAsnGlnLeuCysValLeuHisGluLysThrProValSerAspArgValThrLysCysCysThrGluS
4662 CCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAAC
 504▶ erLeuValAsnArgArgProCysPheSerAlaLeuGluValAspGluThrTyrValProLysGluPheAsnAlaGluTh
4741 ATTCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTGCACTTGTTGAGCTT
 530▶ rPheThrPheHisAlaAspIleCysThrLeuSerGluLysGluArgGlnIleLysLysGlnThrAlaLeuValGluLeu
4820 GTGAAACACAAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGATGATTTCGCAGCTTTTGTAGAGAAGTGCT
 557▶ ValLysHisLysProLysAlaThrLysGluGlnLeuLysAlaValMetAspAspPheAlaAlaPheValGluLysCysC
                                                                   Bsu36I (4968)
4899 GCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTT
 583▶ ysLysAlaAspAspLysGluThrCysPheAlaGluGluGlyLysLysLeuValAlaAlaSerGlnAlaAlaLeuGlyLe
4978 ATAACATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAAAAGCTTATTCATCTG
 609▶ u
5057 TTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCT
                               PvuII (5168)
5136 GTGCTTCAATTAATAAAAAAAAAAAAAAAAAACCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGGTCTGGTGTCAATG
      BamHI (5216)
5215 GGGATCCTCTAGATTGAGTCGACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATT

5294 TTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGT

5373 CTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGAC

                                                                         BbrPI (5
5452 AAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACG

5531 TGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGG

5610 CTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTC

5689 GGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCT

                                                    KasI (5818)
5768 TTGAAAAACACGATAATACCATGGgtaagtgatatctactagttgtgaccggcgcctagtgttgacaattaatcatcgg

5847 catagtatatcggcatagtataatacgactcactataggagggccaccatgtcgactactaaccttcttctctttccta

                                                                          BsiWI (60
5926 cagCTTCGTACCCCGGCCATCAACACGCGTCTGCGTTCGACCAGGCTGCGCGTTCTCGCGGCCATAGCAACCGACGTAC
     ——1▶ SerTyrProGlyHisGlnHisAlaSerAlaPheAspGlnAlaAlaArgSerArgGlyHisSerAsnArgArgTh
6005 GGCGTTGCGCCCTCGCCGGCAGCAAGAAGCCACGGAAGTCCGCCCGGAGCAGAAAATGCCCACGCTACTGCGGGTTTAT
  25▶ rAlaLeuArgProArgArgGlnGlnGluAlaThrGluValArgProGluGlnLysMetProThrLeuLeuArgValTyr
                                      XcmI (6118)
6084 ATAGACGGTCCCCACGGGATGGGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTCGCGCGACGATATCGTCT
  52▶ IleAspGlyProHisGlyMetGlyLysThrThrThrThrGlnLeuLeuValAlaLeuGlySerArgAspAspIleValT
6163 ACGTACCCGAGCCGATGACTTACTGGCGGGTGCTGGGGGCTTCCGAGACAATCGCGAACATCTACACCACACAACACCG
  78▶ yrValProGluProMetThrTyrTrpArgValLeuGlyAlaSerGluThrIleAlaAsnIleTyrThrThrGlnHisAr
                                                                   SphI (6310)
6242 CCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACAATGGGCATGCCTTAT
 104▶ gLeuAspGlnGlyGluIleSerAlaGlyAspAlaAlaValValMetThrSerAlaGlnIleThrMetGlyMetProTyr
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6321 GCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCCGGCCCTCACCC
131▸ AlaValThrAspAlaValLeuAlaProHisIleGlyGlyGluAlaGlySerSerHisAlaProProProAlaLeuThrL
6400 TCATCTTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGCGGTACCTTATGGGCAGCATGACCCCCCA
157▸ euIlePheAspArgHisProIleAlaAlaLeuLeuCysTyrProAlaAlaArgTyrLeuMetGlySerMetThrProGl
                                                                       BspEI (6548)
6479 GGCCGTGCTGGCGTTCGTGGCCCTCATCCCGCCGACCTTGCCCGGCACCAACATCGTGCTTGGGGCCCTTCCGGAGGAC
183▸ nAlaValLeuAlaPheValAlaLeuIleProProThrLeuProGlyThrAsnIleValLeuGlyAlaLeuProGluAsp
6558 AGACACATCGACCGCCTGGCCAAACGCCAGCGCCCCGGCGAGCGGCTGGACCTGGCTATGCTGGCTGCGATTCGCCGCG
210▸ ArgHisIleAspArgLeuAlaLysArgGlnArgProGlyGluArgLeuAspLeuAlaMetLeuAlaAlaIleArgArgV
6637 TTTACGGGCTACTTGCCAATACGGTGCGGTATCTGCAGTGCGGCGGGTCGTGGCGGGAGGACTGGGGACAGCTTTCGGG
236▸ alTyrGlyLeuLeuAlaAsnThrValArgTyrLeuGlnCysGlyGlySerTrpArgGluAspTrpGlyGlnLeuSerGl
6716 GACGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCCCACGACCCCATATCGGGGACACGTTATTTACC
262▸ yThrAlaValProProGlnGlyAlaGluProGlnSerAsnAlaGlyProArgProHisIleGlyAspThrLeuPheThr
6795 CTGTTTCGGGCCCCCGAGTTGCTGGCCCCCAACGGCGACCTGTATAACGTGTTTGCCTGGGCCTTGGACGTCTTGGCCA
289▸ LeuPheArgAlaProGluLeuLeuAlaProAsnGlyAspLeuTyrAsnValPheAlaTrpAlaLeuAspValLeuAlaL
6874 AACGCCTCCGTTCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCCGGCTGCCGGGACGCCCTGCTGCAACT
315▸ ysArgLeuArgSerMetHisValPheIleLeuAspTyrAspGlnSerProAlaGlyCysArgAspAlaLeuLeuGlnLe
                    PshAI (6971)
6953 TACCTCCGGGATGGTCCAGACCCACGTCACCACCCCCGGCTCCATACCGACGATATGCGACCTGGCGCGCACGTTTGCC
341▸ uThrSerGlyMetValGlnThrHisValThrThrProGlySerIleProThrIleCysAspLeuAlaArgThrPheAla
7032 CGTGAGATGATCAGCGGAGCTAATGGCGTCATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCG
368▸ ArgGluMetIleSerGlyAlaAsnGlyValMetAlaLysLeuThrSerAlaValProValLeuThrAlaArgAspValA
                                          SmaI (7150)              SgrAI (7175)
7111 CCGGAGCGGTCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCG
394▸ laGlyAlaValGluPheTrpThrAspArgLeuGlyPheSerArgAspPheValGluAspAspPheAlaGlyValValAr
7190 GGACGACGTGACCCTGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGC
420▸ gAspAspValThrLeuPheIleSerAlaValGlnAspGlnValValProAspAsnThrLeuAlaTrpValTrpValArg
7269 GGCCTGGACGAGCTGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCG
447▸ GlyLeuAspGluLeuTyrAlaGluTrpSerGluValValSerThrAsnPheArgAspAlaSerGlyProAlaMetThrG
7348 AGATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACTTCGTGGCCGAGGA
473▸ luIleGlyGluGlnProTrpGlyArgGluPheAlaLeuArgAspProAlaGlyAsnCysValHisPheValAlaGluGl
                                  RsrII (7457)      SandI (7476)
7427 GCAGGACTGACCGACGCCGACCAACACCGCCGGTCCGACGGCGGCCCACGGGTCCCAG
499▸ uGlnAsp•••
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EXHIBITS

SEQ ID NQ:1 Sequence of plasmid showing the construct containing the reporter gene plus the required enhancer sequence, promoter sequence and associated sequences to allow the plasmid to function according to this invention.

Figure 1. Diagramatic construct of plasmid as defined in SEQ ID NQ:1

Figure 2: Mean changes in serum albumin levels in patients treated with either myosin albumin plasmid vector or CMV albumin plasmid.

Figure 3: Sequential changes in serum albumin levels with time in patients treated with albumin plasmid.

Figure 4: Sequential changes in serum albumin levels with time in patient 3 given two injections at day one and after 27 weeks.

CLAIMS

1. An expression cassette for expressing a human serum albumin into a mammalian cell comprising:
   (i) a cDNA sequence of the human serum albumin,
   (ii) a transcriptional control sequence operably linked to said cDNA sequence.

2. Expression cassette of claim 1 wherein said cDNA sequence of the human serum albumin has the nucleotide sequence set forth in SEQ ID NO:1

3. Expression cassette of claim 1 wherein said cDNA sequence of the human serum albumin is the nucleic acid sequence of about 2.Okb in length obtained by enzymatic digestion by Kpnl and SalI of the plasmid pILMALB5 having the deposit accession number HSRRB HG226

4. Expression cassette of claims 1-3 wherein said transcriptional control sequence comprises a promoter element selected from a viral promoter.

5 Expression cassette of claim 4 wherein said viral promoter is selected from SV4O early and late promoters, the adenovirus major late promoter, the Rous Sarcoma Virus (RSV) promoter, the Cytomegalovirus (CMV) immediate-early promoter and the Majorintermediate-early (MIE) promoter.

7 Expression cassette of claims 4-6 wherein said transcriptional control sequence further comprises at least one enhancer element.

8. Expression cassette of claim 7 wherein said enhancer element is a myosin light chain enhancer.

9. Expression cassette of claim 8 wherein said myosin light chain enhancer is a myosin light chain 1/3 enhancer.

10 Expression cassette of claim 9 wherein said enhancer has the nucleotide sequence set forth in SEQ ID NO:1

11. Expression cassette of claims 7-10 wherein said transcriptional control sequence comprises at the most three enhancer sequences the sequence of which is identical or

selected independently of one another.

12 . Expression cassette of claims 7-11 wherein said enhancer sequence is located upstream of said cDNA sequence of the human serum albumin.

13 . Expression cassette of claim 1 having the sequence set forth in SEQ ID NO:1 which codes for said myosin light chain 1/3 enhancer, said CMV promoter and said cDNA sequence of the human serum albumin.

14 . Expression cassette of claim 1 further comprising a polynucleotide sequence of interest encoding at least one polypeptide of interest distinct from human serum albumin, said polypeptide of interest being co-expressed with human serum albumin in said mammalian cell.

15 Expression cassette of claim 14 wherein said polynucleotide sequence of interest is operably linked to the transcriptional control of said cDNA sequence and comprises at least one ribosome binding site that facilitates translation of messages of any of said polynucleotide seoruence of interest.

16 . A recombinant DNA compound comprising at least one of the expression cassette of claims 1-15.

17 - A compound of claim 16 which comprises at least one nucleotide sequence containing or expressing a selectable marker useful for selecting a cell in which said compound has been introduced.

18 . A compound of claim 17 wherein said nucleotide sequence containing or expressing a selectable marker is an antibiotic resistance gene or an hybrid gene thereof.

19 . A compound of claim 18 wherein said antibiotic is bleomicin or said hybrid is bleomicin:thymidine kinase

20 . A compound of claims 15-18 wherein said recombinant DNA compound is a vector selected from the group consisting in plasmid vector, viral vector or synthetic vector.

21 . A host cell transformed with a compound of claims 15-20.

22 A pharmaceutical composition comprising the compound of claims 15-20.

23 . Use of a cDNA sequence of the human serum albumin for the preparation of a pharmaceutical composition intended for gene transfer.

24 . Use of an expression cassette of claims 1-14 or of a recombinant DNA compound of claims 15-20 for the preparation of a pharmaceutical composition intended for gene transfer.

25 . The use of claims 23-25, wherein said gene transfer is gene therapy for the treatment of the human or animal body.

26 . The use of claims 23-25, wherein said pharmaceutical composition is designed to be administered in a form which can be injected by the intramuscular route.

27 A method for treating a disorder associated with *hypoalbuminemia* in a mammalian organism comprising the step of transferring a vector into tissue or cells of said organism, wherein said vector comprises an expression cassette of claims 1-14 such that human serum albumin is expressed resulting in the treatment of said disorder.

28. Method of claim 27, wherein said disorder is selected in the group consisting of but not restricted to cirrhosis, liver failure, primary and secondary liver cancers, bacterial or viral infections, trauma.

29. Method of treating a human or animal subject, which subject is suffering from a failure or malfunctioning of the liver to produce protein(s) resulting in conditions such as but not limited to hypoalbuminaemia, anaemia, thrombocytopenia and coagulation disorders, which comprises administering to the skeletal muscle of said subject an effective amount of a construct or vector according to the preceding claims encoding said functional protein such as albumin, erythropoietin, thrombopoietin and coagulation factors II,VII,IX and XI . In so doing the host muscle becomes an extra-hepatic source of production of said protein(s).

30. A construct according to claim29 which further comprises an enhancer element.

31. A construct according to any one of the preceding claims wherein the functional protein is erythropoietin.

32. A construct according to any one of the preceding claims wherein the functional protein is thrombopoietin.

33. A construct according to any of the preceding claims where the gene encoding for endorphins, insulin, antisense gene for angiotensin, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), angiostatin, endostatin, or thrombospondin is included.

34. A construct according to any of the preceding claims where the plasmid is encoded for interferon, or the gene encoding the envelope protein or core protein of viruses such as, but not exclusively, hepatitis B & C or HIV.

35. A construct according to any of the preceding claims where the plasmid is encoding specific tumour associated antigens including, but not exclusively, carcinoembryonic antigen (CEA), CA125, CA19.9 or prostate specific antigen (PSA).

36. A construct according to any of the preceding claims where the plasmid is encoding amino acid sequences or proteins produced or related to the low density lipoprotein (LDL), very low density lipoprotein (VLDL) or high density lipoprotein (HDL) receptors.

37. A construct according to any of the preceding claims where the plasmid is encoding the gene for Endothelial Nitric Oxide Synthetase (ENOS), or Granulocyte Colony Stimulating Factor (GCSF), Macrophage Colony Stimulating Factor (MCSF) or Granulocyte Macrophage Colony Stimulating Factor (GMCSF).

38. A construct according to any of the preceding claims for use in a method of treatment of the human or animal body.

The Patent Office

INVESTOR IN PEOPLE

Application No:	GB 9930891.8	**Examiner:**	L.V.Thomas
Claims searched:	1-26 and 30-38	**Date of search:**	20 September 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, BIOSIS, MEDLINE, CAS-ONLINE, SCISEARCH, EMBASE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP 0771874 A2	(STATE OF ISRAEL) see particularly p.3 ll.15-52	1,2
E, X	WO 99/66054 A2	(GENZYME) see particularly pp.1-4, 16, 29-30 and 39	1,14,16,21 -25, 31,38
X	WO 93/03164 A1	(RHONE-POULENC ET AL.) see p.4 l.18 - p.5 l.24 and p.8 ll.16-34	1,2
X	WO 92/22635 A1	(UNIV. OF CONNECTICUT ET AL.) see p.2 l.25 - p.4 l.6 and pp.11-13	1,2
X	Transgenic Research 1994, 3(3), pp.141-151 Barash et al. - see the abstract and "Materials and methods" on pp.142-144		1,2

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.